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THE EFFECT OF SALINITY UPON PHOTOTAXIS AND GEOTAXIS IN A LARVAL CRUSTACEAN

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Vertical movements by pelagic larvae of marine benthic invertebrates are directed by light and gravity. The spatial orientations of these responses are known to be affected by light intensity, temperature, hydrostatic pressure, feeding, and salinity (reviewed by Forward, 1976a). This last parameter, which is highly variable in coastal plain estuaries, can alter both phototactic and geotactic behavior.

A negative phototaxis can be induced in normally photopositive larvae of various estuarine organisms by a sudden exposure to low salinities. The salinity change necessary for such a phototactic reversal may range from 4.5% to 66% dilution of seawater (Edmondson and Ingram, 1939; Lynch, 1949; Lyon, 1906; Ranade, 1957). Furthermore, Edmondson and Ingram (1939) found that barnacle nauplii regained positive phototaxis in ten minutes after a salinity decrease. Evidence that an increase in salinity alters the sign of phototaxis is unreported.

A negative geotaxis enables larvae to remain up in the water column in the absence of light by directed swimming which compensates for a tendency to sink (Sulkin, 1973). The depth in the water column at which larvae are found, however, is related to developmental stage. Generally, later larval stages remain lower than earlier stages (*e.g.*, Bousfield, 1955; Carriker, 1951; Lynch, 1947; Sandifer, 1975; Sulkin, 1973).

Work with salinity discontinuities in nature (Grindley, 1964) and in the laboratory (Harder, 1968; Lance, 1962; Lyster, 1965; Roberts, 1971; Scarratt and Raine, 1967), as well as laboratory experiments with fluctuating salinities (Haskin, 1964; Hughes, 1969; Hughes and Richard, 1973), have shown that a downward movement is the common response to a salinity decrease. Likewise, an increase in salinity will induce an upward movement (Haskin, 1964; Hughes and Richard, 1973). Little attempt, however, has been made to determine whether such movements result from a response to light and/or to gravity.

The present study examines the effect of sudden salinity changes upon phototaxis and geotaxis in larvae of the brachyuran crab *Rhithropanopeus harrisii* (Gould). This species was chosen for study because its larvae occur in coastal plain estuaries, where they are subjected to natural salinity variations. In addition, much is known about the larvae: the effects of salinity on larval development (Costlow, Bookhout, and Monroe, 1966), osmoregulatory ability (Kalber and Costlow, 1966), the ontogeny of phototaxis (Forward, 1974; Forward and Costlow, 1974), the shadow response (Forward, 1976b), the effect of temperature on phototaxis and geotaxis (Ott and Forward, 1976), polarotaxis (Via and Forward,

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1975) and occurrence of the larvae in estuaries (Bousfield, 1955; Chamberlain, 1962; Pinschmidt, 1963; Sandifer, 1975).

The results from the study indicate that sudden salinity changes alter phototaxis and geotaxis by Stage I and IV zoeae, and it is suggested that these behaviors contribute to vertical movements within a stratified estuary.

Materials and Methods

Ovigerous female specimens of *Rhithropanopus harrisii* (Gould) which were collected from the Neuse River in eastern North Carolina, from May until August, were maintained in either 10 or 20‰ filtered sea water. Upon hatching, larvae were cultured using techniques similar to that of Costlow *et al.* (1966) in 10 or 20‰ filtered sea water (acclimation salinities) and maintained at 25° C on a 12L:12D cycle in a Sherer Controlled Environment Chamber (Model CEL-44). Larvae were transferred daily to clean finger bowls containing fresh sea water and were fed newly hatched *Artemia salina* nauplii.

The light source for the phototaxis experiments was a La Belle slide projector fitted with a 300 W tungsten bulb. Light was filtered by two hot mirrors (Baird Atomic) and a Corning No. 1–75 filter in order to reduce heat, and was then filtered to 500 nm with a thin film absorption filter (half band pass 7.4 nm; Ditric Optics, Inc.). This wavelength was chosen for experimentation since a previous study indicated that this species shows maximal sensitivity at this spectral region (Forward and Costlow, 1974). There is no evidence that larvae of *R. harrisii* exhibit color dances or other changes in swimming orientation with wavelength, as has been reported in some crustaceans (*e.g.*, Dingle, 1962). Light intensity was regulated by neutral density filters (Ditric Optics, Inc.) and was measured by a radiometer (YSI model 65).

Phototactic behavior of larvae of *R. harrisii* was monitored in both a horizontal and a vertical plane upon sudden exposure to a range of different salinities. Experimentation using a horizontally-directed light source was performed with a 15 × 3 × 3 cm leucite cuvette, which was divided into five equal sections along the longitudinal axis. The sections were separated by thin slides constructed so that all could be moved vertically in unison.

The test chamber with the vertically directed light source was a 45 × 8 × 7.5 cm upright rectangular leucite cuvette. Light entered the chamber from either above or below by means of an appropriately positioned mirror. The cuvette was marked into ten vertical sections, each 3.8 cm in height. A 20 W daylight fluorescent light was mounted vertically behind the chamber to aid in viewing larval positions at the termination of stimulation.

During larval development *R. harrisii* has four zoeal stages which are free-swimming in the plankton and are phototactic. The subsequent megalopa stage probably settles out of the plankton and is unresponsive to light (Forward, 1974; Forward and Costlow, 1974). Thus only Stage I and IV zoeae were tested, since they are responsive to light and any ontogeny in responses should be apparent. For all experiments larvae were light-adapted under a 60 W incandescent bulb in addition to fluorescent room lights, for at least one-half hour prior to testing. Experimentation was performed within a six-hour period which began three hours after the onset of the light phase in order to avoid any complications due to a bio-

logical rhythm in either phototaxis or salinity responses. Testing was performed at room temperature (22–25° C). Even though this temperature range does deviate from that in the culture cabinet, the change is insufficient to alter either phototaxis or geotaxis (Ott and Forward, 1976). For each phototactic determination with the horizontal and vertical light source approximately 60 larvae from at least three separate females were used. Salinities for most experiments were measured with an American Optics refractometer (accuracy 1.0‰). However, the salinities of the solutions used for determining the salinity threshold for a negative phototaxis were measured with an osmometer (Model 65–31, Advanced Instruments, Inc.; accuracy 0.2‰), the calibration for which was established with an induction salinometer (Hytech Model 6220).

The general procedure for horizontal stimulation consisted of pipetting ten larvae into the center section of the cuvette filled with sea water of the experimental salinity. After 50 seconds in total darkness, the slides separating the sections were raised, and 10 seconds later the light stimulus was applied for a duration of 30 seconds, after which time the slides were lowered. This stimulus time was chosen because preliminary testing indicated that it was sufficient for initiating a clear response, but was insufficient to induce an apparent total (100%) response. The distribution of larvae among the five sections was then recorded. Thus, larvae were stimulated with light one minute after exposure to sea water of the experimental salinity, and the light stimulus was terminated 1.5 minutes after exposure to the sea water. Controls of random swimming in darkness were also run following a similar procedure, except that larvae remained in darkness for the entire trial. For determining phototactic responses, only larvae located in the section nearest the light source were considered to be displaying positive phototaxis, while only larvae in the furthest section were said to show a negative response. Levels of responsiveness under different conditions were statistically compared by determining a Z-statistic for testing the difference between two proportions (Walpole, 1974) and significant differences tested at the 0.05 level.

Initially, the phototactic responses of larvae acclimated to 20‰ sea water and stimulated in a horizontal plane were measured for stimulus intensities of 1.93 to 1.93×10^{-5} Wm^{-2}, as well as a control in darkness. The experimental salinities were 5, 20, and 40‰. To establish the smallest decrease in the salinity from the acclimation salinity that would evoke a reversal in the sign of phototaxis from positive to negative, larvae were acclimated to 10 or 20‰ sea water. They were then exposed to salinity decreases of 0.8, 1.1, 1.3, 1.5, 1.7, 2.0‰, and levels of positive and negative phototaxis were observed upon stimulation at 0.19 Wm^{-2}, an intensity which at the acclimation salinity provokes a positive response.

Further experiments measured the time for a positive phototaxis to be re-established. Larvae were exposed to salinity decreases equal to or slightly greater than threshold values for inducing a reversal in phototactic sign. For comparison, Stage I zoeae (acclimation salinity 20‰) were subjected to 5‰ and a similar time measured. The testing procedure was similar to that previously delineated, in that the light stimulus ($I = 0.19$ Wm^{-2}) commenced one minute after exposure to the salinity change. Then, with the light remaining on, the distribution of larvae in the test cuvette was noted 30 seconds later and thereafter at one minute intervals until a 45–55% response level of positive phototaxis was established. This level

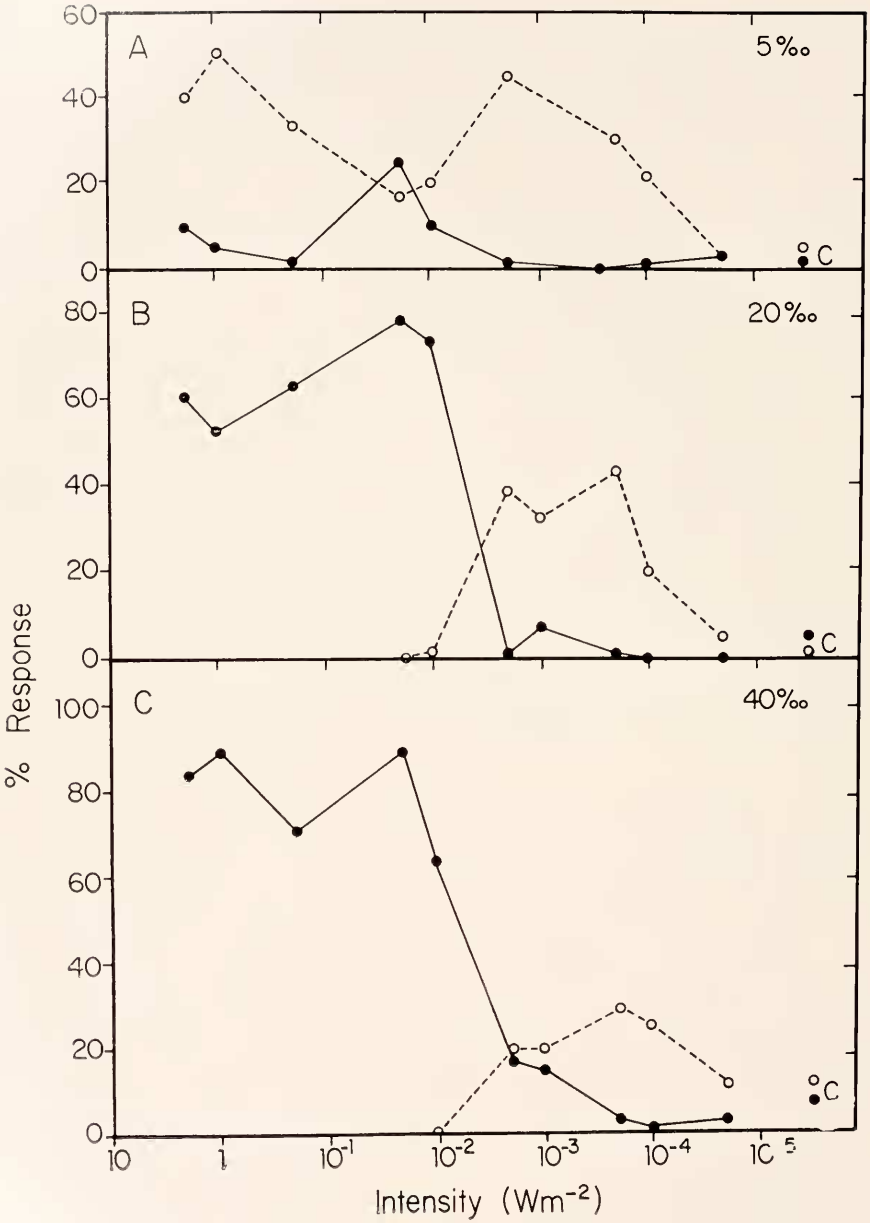

FIGURE 1. Stage I: per cent response (ordinate) of positive (solid dot-solid line) and negative (open dot-dashed line) phototaxis to various stimulation intensities of 500-nm light (abscissa) upon sudden exposure to salinities of 5‰ (A), 20‰ (B), and 40‰ (C) for larvae acclimated to 20‰. Random swimming in the positive (solid circle) and negative (open circle) direction was determined with no stimuli present. Sample size for each point was 60 individuals. In 5‰ sea water (A), the level of positive phototaxis at all light intensities above 10^{-3} Wm^{-2} is significantly less than that in 20‰. The level of negative phototaxis present at low light

of responsiveness was chosen to indicate a total return of responsiveness, since it is a level similar to that shown by the larvae before they experience the salinity change. The amount of time needed to reach this "total" recovery and a level of responsiveness that was one half this value (50% recovery) were determined.

Experiments done in the vertical plane were designed to test the effect of sudden salinity changes upon vertical distributions. Larvae were tested in darkness and upon vertical stimulation with a light intensity of 1.5×10^{-1} Wm^{-2} as measured at the surface of the column nearest to the stimulus source (*i.e.*, bottom of column with light from below, top of chamber with light from above). This intensity induces a positive phototaxis at acclimation salinities when the larvae are stimulated horizontally. Larvae were acclimated to 20‰ sea water, and a range of seven salinities were tested: 5, 10, 15, 20, 25, 30, and 40‰.

For testing, larvae were pipetted 10 cm down into the cuvette filled with the experimental salinity sea water. They were then either subjected to the light stimulus or allowed to remain in darkness for two minutes, after which time the fluorescent light mounted behind the chamber was turned on, and the distribution of larvae recorded. Preliminary studies indicated that this stimulus duration gave larvae more than sufficient time to reach equilibrium positions. Distributions in light and darkness at either the top or bottom section of the column at each salinity were compared statistically by means of a 2×2 contingency test in which larvae were assigned to either of two groups; top or bottom and all others. Differences were tested at the 0.05 level. Negative geotaxis was considered vertical movement up in the water column in darkness, while positive geotaxis was the opposite.

The passive sinking rates of larvae were measured in 5, 10, 15, 20, and 40‰ at room temperature. Larvae were anesthetized by placing a few drops of propylene phenoxytol in a well slide containing 10 to 15 larvae in water at the acclimation salinity (for methods see Forward, 1976a). Larvae were gently placed in the upright cuvette filled with the experimental salinity sea water and allowed to sink at least 7.6 cm (two sections) in order to reach terminal velocity. The time it took larvae to traverse a subsequent 3.8 cm was measured by a stopwatch. Sinking rates were measured for at least 20 separate animals under each salinity condition. In addition, descent rates and swimming behavior of unanesthetized larvae (20‰ acclimation salinity) were examined in 5, 10 and 15‰ salinity within the vertical column with light entering from above. A procedure similar to that for anesthetized larvae was followed, and the light stimulus intensity was 0.15 Wm^{-2}. Using the Student's *t*-test, mean rates for normal and anesthetized larvae at each salinity were tested for significant differences at the 0.05 level.

Results

Horizontal testing

The pattern of phototaxis by Stage I and IV zoeae of *Rhithropanopeus harrisii* is altered upon exposure to low salinity sea water. Stage I zoeae sub-

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intensities (less than 10^{-2} Wm^{-2}) in 40‰ sea water is significantly greater than control levels of random swimming but is significantly less than similar responses in 20‰. At light intensities above 10^{-2} Wm^{-2}, the level of positive phototaxis in 40‰ is significantly greater than that in 20‰ except at 0.2 Wm^{-2}, where the responses are not significantly different.

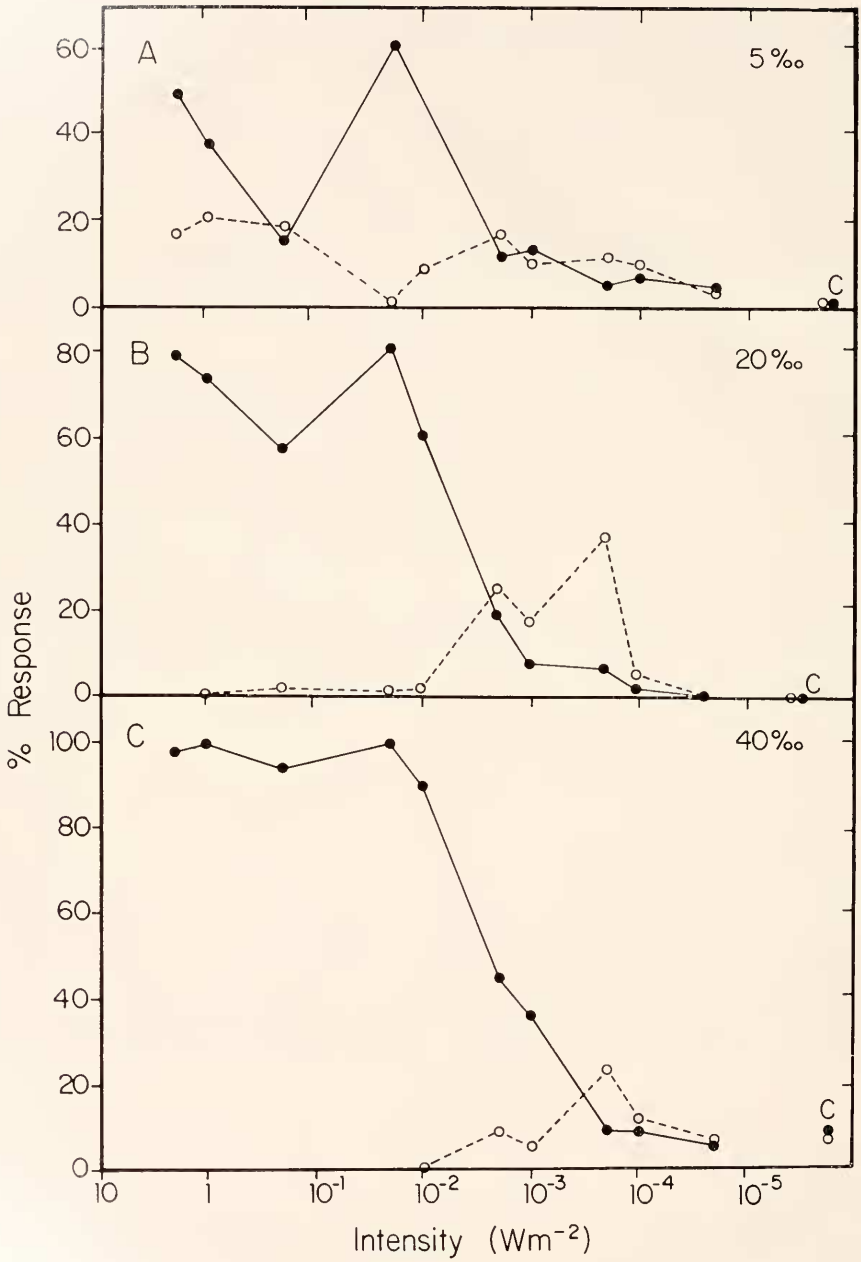

FIGURE 2. Stage IV: per cent response of positive and negative phototaxis to various intensities of 500-nm light upon sudden exposure to salinities of 5‰ (A), 20‰ (B), and 40‰ (C) for larvae acclimated to 20‰. The figure is plotted as described for Figure 1. Sample size for each point was 60. At the intensity (1.9×10^{-4} Wm^{-2}) that induces the greatest

jected to 5‰ (Fig. 1A) display a pronounced negative phototaxis at light intensities above 10^{-3} Wm^{-2}, while the normal positive response, which is prominent at the acclimation salinity of 20‰ (Fig. 1B), is suppressed. Such a dramatic reversal in phototaxis is not observed upon exposure to 40‰ sea water (Fig. 1C), where the response pattern is similar to that at 20‰. The level of positive phototaxis to intensities above 10^{-2} Wm^{-2}, however, is generally greater for larvae in 40‰.

Stage IV zoeae, subjected to 5‰ (Fig. 2A), also show greater negative phototaxis at high light intensities (above 10^{-2} Wm^{-2}) than those observed at 20‰ (Fig. 2B). In addition, while levels of positive phototaxis are not as great as those at 20‰, a positive response is still present at intensities above 10^{-2} Wm^{-2}. Like Stage I, the pattern of photoresponses of Stage IV zoeae exposed to 40‰ is similar to that seen at the acclimation salinity (Fig. 2C). The negative phototaxis at low light intensities, however, is suppressed, and the level of positive phototaxis at higher intensities is greater.

Since lowering the salinity generally reverses the sign of phototaxis from positive to negative at stimulus intensities above 10^{-2} Wm^{-2}, further experiments established the amount of salinity decrease from the acclimation salinity necessary for this change. The threshold value for inducing a reversal in phototactic sign is considered to be that salinity which produces a negative response level significantly greater than the control level in the acclimation salinity. When larvae are acclimated to 20‰ salinity, the threshold values are: Stage I zoeae 1.1‰, and Stage IV zoeae 2.0‰ (Fig. 3A, B). Upon acclimation to 10‰ the threshold value is 1.3‰ for both Stages I and IV zoeae (Fig. 3C, D). Since these values are similar, the threshold is apparently independent of the acclimation salinities and larval age.

The length of time for recovery of a positive phototaxis upon lowering the salinity is also independent of the acclimation salinity and developmental stage, as well as the magnitude of the salinity decrease (Table I). A total recovery occurs in approximately 5.5 minutes under all conditions, with a 50% recovery apparent in 1.5–3.2 minutes. Clearly the suppression of a positive phototaxis is a short-term phenomenon.

Throughout the experiments with horizontal stimulation, qualitative observations were made of larval behavior. While elimination of a vertical component in responses had been sought, apparently this could not be totally avoided. Larvae exposed to 5‰ were consistently found on the bottom of the cuvette, while larvae in 40‰ sea water swam freely. To quantify these observations and investigate the interaction of phototactic and geotactic components of a response to salinity stimuli, tests were performed in a vertical column.

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negative response in 20‰ (B), the response level in 5‰ (A) is significantly less. At higher intensities (above 10^{-2} Wm^{-2}), the level of negative phototaxis is significantly greater than that in 20‰. Levels of positive phototaxis at intensities above 10^{-2} Wm^{-2} are significantly greater than the dark control, yet significantly less than those in 20‰. In 40‰ (C) sea water, the only intensity at which a level of negative phototaxis is significantly greater than the control is 1.9×10^{-4} Wm^{-2}, but the level at this intensity does not differ significantly from that in 20‰. At intensities of 10^{-3} Wm^{-2} and greater, the level of positive phototaxis in 40‰ is significantly greater than that in 20‰.

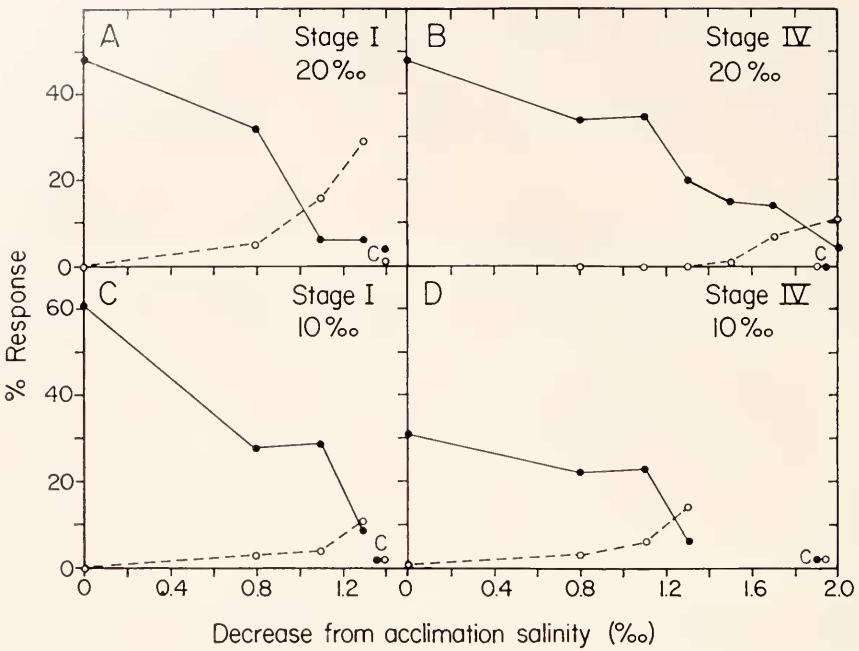

FIGURE 3. The minimum salinity decrease necessary for reversing the sign of phototaxis. Per cent response (ordinate) of positive (solid dot-solid line) and negative (open dot-dashed line) phototaxis to a stimulus of 0.19 Wm^{-2} 500-nm light upon various salinity decreases from the acclimation salinity (abscissa) by Stage I (A) and IV (B) zoeae acclimated to 20‰ and Stage I (C) and IV (D) zoeae acclimated to 10‰. Random swimming in the positive (solid circles) and negative (open circles) directions was determined with no stimulus present. The average sample size for each point was: (A) = 77; (B) = 65; (C) = 95; and (D) = 80.

Vertical testing

Experiments with light entering the column from above resembled the downward-directional aspect of natural light conditions and examined the effect of salinity changes on vertical distributions under this irradiation regime. Those with light entering the column vertically from below had two objectives. The first was to further clarify the role of a negative phototaxis in establishing vertical distributions upon sudden exposure to salinities below the acclimation salinity. The second was to determine whether the positive phototaxis or negative geotaxis is the dominant behavioral response during the ascent observed upon exposure to salinities above the acclimation salinity.

The vertical distribution of larvae is age-dependent. In acclimation salinity sea water, Stage I zoeae (Fig. 4) are dispersed throughout the vertical column, although slightly more are present in the lower half (light from above 67%, darkness 68%; no significant difference). Stage IV zoeae, however, are positioned lower in the column (Fig. 5). In darkness, essentially all larvae (97%) were found in the lower half of the column, while 85% of those irradiated from above were so distributed.

TABLE I

Times to recover positive phototaxis (total recovery) and responsiveness at one-half this value (50% recovery) to 500-nm light of intensity 0.19 Wm^{-2} by Stage I and IV zoeae acclimated to 10 and 20‰ sea water and exposed to lower salinities (Salinity). Times were measured from the initial exposure to the salinity decrease, and response (%) indicates the level of positive phototaxis at the time recorded. Average n was 76.

	Total Recovery			50% Recovery	
	Salinity (‰)	Response (%)	Time (min)	Response (%)	Time (min)
20‰ acclimation					
Stage I	18.7	53	5.5	27	2.9
	5.0	53	5.0	27	3.2
Stage IV	17.5	54	5.5	27	1.5
10‰ acclimation					
Stage I	8.7	45	5.5	23	2.0
Stage IV	8.7	47	5.5	24	2.7

Experiments indicate that the vertical distribution of larvae is altered by salinity. For Stage I zoeae (Fig. 4), when the distributions in the top and bottom sections of the column are compared between the two conditions of darkness and irradiation from above, there is no statistical difference. For each test at salinities less than the acclimation salinity, Stage I zoeae are found lower in the column as compared to distributions in 20‰. This is most clearly observed at the lowest test salinity (5‰), in which 93% of the larvae in light and 92% in darkness are located at the bottom of the column (Fig. 4). Exposure to salinities above the acclimation salinity causes an upward movement. At 40‰, all larvae are positioned within the upper third of the column with approximately 97% in light and 100% in dark found in the uppermost 3 cm.

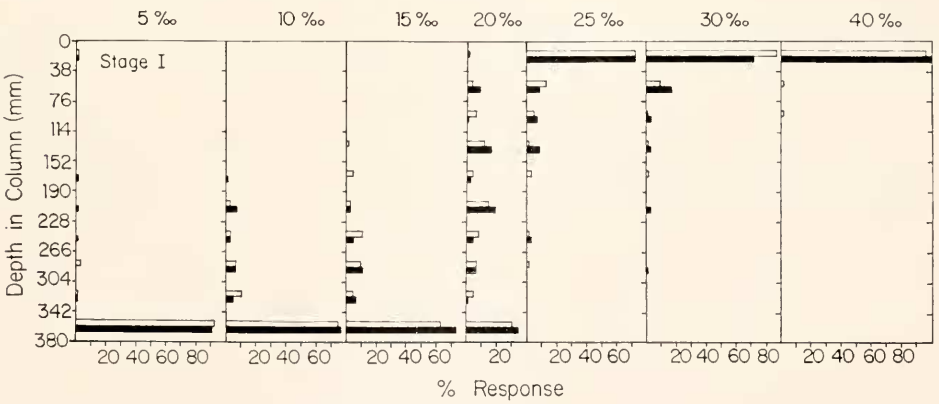

FIGURE 4. Stage I: percentage distribution of larvae (abscissa) according to depth in column (ordinate) upon stimulation by 0.15 Wm^{-2} 500-nm light direction from above (open bar) or in total darkness (solid bar) when acclimated to 20‰ and exposed to salinities between 5 and 40‰. Sample size for each condition was 60 individuals.

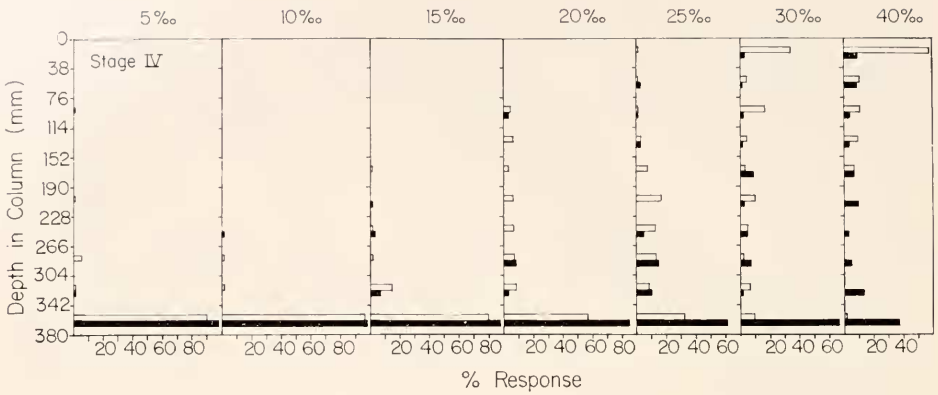

FIGURE 5. Stage IV: percentage distribution according to depth upon stimulation by 0.15 Wm^{-2} 500-nm light directed from above or in total darkness when acclimated to 20‰ and exposed to salinities between 5 and 40‰. The figure is plotted as described for Figure 4. Sample size for each condition was 60.

The percentage of Stage I zoeae positioned on the bottom of the column upon exposure to various salinities when irradiated from below is given in Table II. Due to an apparent positive phototaxis, 87% of the larvae are located on the bottom in 20‰ sea water (acclimation salinity). This percentage declines upon exposure to lower salinities as larvae move up in the water column. This ascent must be due to a negative phototaxis, since in darkness larvae descend at these salinities (Fig. 4). In 25, 30, and 40‰ (Table II), more than 90% of the larvae occur at the bottom. This indicates that at these salinities a positive phototaxis is the dominant response, as a negative geotaxis occurs in darkness (Fig. 4). In addition, this is consistent with horizontal testing at 40‰ (Fig. 1C), in which the levels of positive phototaxis at higher light intensities are significantly greater than those at 20‰ (Fig. 1B). Thus, for Stage I zoeae, the dominant behavioral response upon exposure to low salinities is a negative phototaxis, while at higher salinities it is a positive phototaxis.

TABLE II

Percentage of larvae in bottom 4 cm of vertical column upon stimulation by 0.15 Wm^{-2} 500-nm light directed from below when exposed to salinities between 5 and 40‰. Acclimation salinity was 20‰; sample size for each salinity condition was 60 individuals.

Salinity (‰)	Percentage of larvae on bottom	
	Stage I	Stage IV
5	40	85
10	26	82
15	58	67
20	87	95
25	92	97
30	98	100
40	97	100

Exposure of Stage IV zoeae to salinities less than 20‰ (Fig. 5) results in a downward movement in both darkness and with illumination from above. The distributions in light and darkness are not significantly different. At higher salinities of 30 and 40‰, a pronounced upward movement occurs only in the light.

The negative phototaxis present at 5, 10, and 15‰ (Fig. 2A) contributes little to the vertical distribution of Stage IV zoeae (Fig. 5), since upon irradiation from below most of the larvae are found on the bottom of the column (Table II). In contrast, greater than 97% of the larvae exposed to salinities above the acclimation salinity are located on the bottom (Table II). Therefore, the dominant response by Stage IV zoeae upon exposure to low salinities is a positive geotaxis, while at higher salinities an increased level of positive phototaxis accounts for the upward movement of larvae when irradiated from above.

Anesthetized larvae sink in both high and low salinity sea water (Table III). Therefore, those larvae found up in the water column must be maintaining their position by active swimming. The larger Stage IV zoeae exhibit mean sinking rates approximately three times greater than those for Stage I. In general, sinking rates for a given larval stage increase with decreasing salinity, due to the associated decrease in density of the sea water.

Descent rates of unanesthetized Stage I and IV zoeae in 5, 10, and 15‰ in sea water with light directed from above are generally significantly greater than passive sinking rates, indicating that the descent involves active downward swimming (Table III). The exception to this is Stage IV zoeae in 15‰, where even though the mean velocity of unaesthetized larvae increased 15% over that for passive sinking, no significant difference between mean rates was obtained due to high variance. Although only mean speeds are presented in Table III, the distribution of descent speeds shown by unanesthetized larvae indicates that some larvae at each lower salinity descend by passive sinking. Furthermore, while rates of both unanesthetized Stage I and IV zoeae in 10‰ were greater than those in 15‰, possibly due to the greater salinity stimulus, rates in 5‰ were less than

TABLE III

Descent rates (cm/sec) by anesthetized and unanesthetized (normal larvae) upon sudden exposure to different salinities. Larvae were acclimated to 20‰. Velocities were measured in the vertical plane at room temperature (23–25° C). Normal larvae were irradiated from above with 500-nm light of 0.15 Wm^{-2} intensity. The mean ($\bar{x}$), standard deviation (s.d.) and sample size (N) are shown. The asterisk indicates that mean sinking rate for normal larvae at a particular stage and salinity is significantly greater than the mean rate for anesthetized larvae in the same salinity.

Salinity (‰)	Stage I						Stage IV					
	Normal			Anesthetized			Normal			Anesthetized		
	$\bar{x}$	s.d.	N	$\bar{x}$	s.d.	N	$\bar{x}$	s.d.	N	$\bar{x}$	s.d.	N
5	0.46*	0.16	24	0.30	0.04	27	1.32*	0.70	23	0.98	0.09	21
10	0.66*	0.21	23	0.29	0.02	25	1.93*	0.89	23	0.81	0.10	23
15	0.54*	0.44	24	0.33	0.05	23	1.06	0.70	24	0.92	0.07	25
20	—	—	—	0.31	0.04	23	—	—	—	0.78	0.08	28
40	—	—	—	0.21	0.02	25	—	—	—	0.60	0.01	28

at 10‰. This may be caused by the shock experienced by the larvae when subjected to a 15‰ salinity decrease, rather than an active swimming response.

Discussion

Phototaxis by larvae of *Rhithropanopeus harrisii* is modified by a change in salinity. Upon exposure to lower salinities at light intensities above 10^{-2} Wm^{-2}, the positive response is suppressed while the level of the negative response increases. These changes in responsiveness are more pronounced in Stage I than Stage IV zoeae. A similar reversal in the sign of phototaxis from positive to negative upon exposure to low salinity sea water has been observed in larvae of the grass shrimp *Palaemonetes* (Lyon, 1906) and the barnacle *Balanus amphitrite* (Edmondson and Ingram, 1939).

A decrease in salinity of only 1.1‰ from the acclimation salinity will induce this reversal in the sign of phototaxis in Stage I zoeae acclimated to 20‰. The minimum salinity decrease necessary for inducing this behavior in Stage IV zoeae and in larvae acclimated to 10‰ is comparable (within 1.0‰). Since these values are similar, the salinity change threshold apparently does not depend upon acclimation salinity or developmental age. Instead, larvae are responding to the salinity decrease from that particular acclimation salinity and not to an absolute salinity.

These results indicate a high sensitivity to salinity in a euryhaline brachyuran larva (Costlow *et al.*, 1966), which compares to that by larvae of the stenohaline polychaete *Polydora pulchra*. For this polychaete, a salinity decrease of 1.5‰ from that in its natural environment (34‰) induces a negative phototaxis (Ranade, 1957). According to Thorson (1964), larvae of intertidal species require a much greater salinity decrease to reverse the sign of phototaxis than those from subtidal species. Indeed, this is true for several species, such as larvae of *Balanus amphitrite,* in which a 50% dilution of seawater is necessary to initiate a negative phototaxis (Edmondson and Ingram, 1939). This generalization, however, is questionable, since *R. harrisii* is a low intertidal species (Bousfield, 1955; Pinschmidt, 1963; Smith, 1967).

The negative phototaxis that is induced in larvae of *R. harrisii* by lowering the salinity is clearly a short-term response. A positive phototaxis is totally recovered in 5.5 minutes, with a 50% return in 1.5–3.2 minutes. Whether exposed to a 1.3‰ or a 15‰ salinity decrease, these times are similar. Thus, the return of a positive phototaxis seems independent of both the developmental stage and the amount of salinity change beyond the threshold value. Similar times have been observed for nauplii of *B. amphitrite*, which upon exposure to 50% sea water regain a positive phototaxis in ten minutes (Edmonson and Ingram, 1939). The short-term recovery of the positive phototaxis upon a sudden change in salinity may possibly be a general characteristic of pelagic larvae.

Stage IV zoeae are positioned lower in the experimental water column than Stage I in darkness and under overhead light at the acclimation salinity. This general pattern has been predicted (Ott and Forward, 1976) and observed in the field (Sandifer, 1975) for larvae of *R. harrisii*. A deeper net distribution of later stages has also been noted for estuarine larvae of the crabs *Leptodius floridanus* and *Panopeus herbstii* (Sulkin, 1973; 1975), the shrimps *Penaeus duorarum* (Hughes, 1969) and *Macrobarchium acanthurus* (Hughes and Richard, 1973),

and the barnacle *Balanus improvisus* (Bousfield, 1955). The mechanism underlying these distribution patterns is unknown.

Previous laboratory studies indicate that downward movement is induced in zooplankton upon exposure to lower salinity sea water. This has been observed in 17 copepod species (Grindley, 1964; Harder, 1968; Lance, 1962), the branchiopod *Artemia salina* as well as nauplii of the cirripeds *Pollicipes polymerus* and *Balanus tintinnabulum* (Harder, 1968), and larvae of the decapods *Macrobrachium acanthurus* (Hughes and Richard, 1973), *Porcellana longicornis* (Lance, 1962), *Paguris longicarpus* (Roberts, 1971), and *Homarus americanus* (Scarratt and Raine, 1967). Generally two mechanisms of response have been observed: directed downward swimming and passive sinking. Whether the downward swimming is due to a positive geotaxis and/or negative phototaxis had not been determined. In the present study, the components involved have been identified.

Although the vertical distributions of both Stage I and IV zoeae of *R. harrisii* in light and darkness upon exposure to salinities below the acclimation salinity are similar, the behavioral response mechanisms underlying their distribution are not uniform. The descent by both zoeal stages in darkness is due to a postive geotaxis generally involving active downward swimming. Although a positive geotaxis can contribute to the descent under overhead light, the dominant behavioral response for Stage I zoeae is a negative phototaxis, since the larvae ascend upon irradiation from below. In contrast this ascent is much weaker for Stage IV zoeae, so a negative phototaxis contributes little to downward movement under overhead light, and the positive geotaxis is the main behavioral response.

The ascent by larvae upon an increase in salinity does not result from floating due to the increased density of water, since anesthetized larvae sink in the higher salinities. Stage I zoeae are positioned high in the water column due to a negative geotaxis in darkness. Again, the geotaxis can contribute to movements under overhead light, but the primary behavioral response is a positive phototaxis, since the larvae descend upon irradiation from below. In contrast, Stage IV zoeae only show a pronounced ascent due to a positive phototaxis when irradiated from above. In darkness, a very weak upward movement is observed, which implies that this zoeal stage will only ascend upon exposure to a salinity increase during the day.

In coastal plain estuaries usually a moderate amount of vertical stratification by salinity exists, in which an upper layer of low salinity sea water is separated from a lower layer of high salinity sea water by a region of water characterized by a salinity gradient (Pritchard, 1967). Pinschmidt's (1963) study in the Newport River estuary (North Carolina) supports this generalization, since he demonstrated that at the two stations where *R. harrisii* larvae were most abundant, mean salinity differences between surface and bottom water were 2.3‰ during high tide and 1.5‰ for low tide. A more recent unpublished study by Thomas Cronin (Duke University Marine Laboratory) indicates that in areas of this estuary where the larvae occur, salinity differnces between the surface and bottom can be as high as 9‰, but the average is around 5‰. Furthermore, in the Neuse River estuary (North Carolina), monthly salinity readings over the course of three summers from two stations in the vicinity of the collecting site for the present study indicate that this region is also stratified, with a mean salinity differential between surface and bottom of 1.8‰ (Hobbie and Smith, 1975). Thus salinity changes throughout the

water column do exist in areas where *R. harrisii* larvae occur and are sufficient to evoke behavioral responses.

The predicted general behavior of larvae confronted with salinity variations is as follows. Larvae entering the upper layer of reduced salinity sea water will experience a decrease in salinity and consequently respond by a downward movement due to a positive geotaxis and/or negative phototaxis and therefore descend into sea water of the original salinity. A decrease of only 1–2‰ will induce the negative photoaxis. While a positive geotaxis is clearly induced by a 5‰ change, it seems probable that the thresholds for this response are similar to those for the negative photoaxis. As larvae regain normal phototactic responsiveness at a new salinity in 5.5 minutes, a salinity decrease would have to be experienced over a very short time span.

Although the return to a positive phototaxis occurs quickly, there is sufficient time for larvae to negotiate a substantial portion of the water column. Mean descent rates of larvae acclimated to 20‰ and experiencing a 5–10‰ salinity decrease in the presence of light are 0.6 cm/sec for Stage I and 1.5 cm/sec for Stage IV. Within the span of time allowing for a 50% to full recovery of the positive phototaxis, it is calculated that Stage I zoeae can transverse a vertical distance of 1–2 m and Stage IV 1.4–5 m. In the region of two estuaries where larvae are abundant, mean depths at low tide are 3.5–5 m (Bousfield, 1955; Pinschmidt, 1963). The short-term recovery indicates that if a larva cannot lose the negative response by escaping from the salinity region which induced it, the response will be lost over time, thereby allowing the animal to acclimatize to a new salinity and resume normal behavior.

Alternately, larvae could move down into sea water of a higher salinity than that to which they are acclimatized. In this case, they swim upwards due to a positive phototaxis and/or a negative geotaxis. Therefore, these behavioral responses to increases and decreases in salinity can act as a negative feedback system to keep larvae within the region of acclimatization salinity water.

A limitation of the present study is that only sudden changes in salinity were considered. At present nothing is known about rates of salinity change, which are necessary to initiate these behavioral responses. In addition, the physiology of these responses is relatively unstudied. For example, the site of salinity detection is not known. Roberts (1971) demonstrated that it is not on the uropods or antennae of larvae of *Paguris longicarpus,* as amputated animals showed identical salinity responses as nonamputees. Also, the mechanism whereby a salinity decrease reverses the sign of phototaxis, and even the site of gravity reception for geotaxis, are unknown. As zoeae possess no functional statocyst (Prentiss, 1901), perhaps the receptors are located in the numerous spines covering the carapace (Foxon, 1934). The need for suitable studies on these aspects of larval physiology is evident.

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Summary

1. Experiments were conducted to determine the effect of salinity on phototaxis and geotaxis by Stages I and IV zoeae of the crab, *Rhithropanopeus harrisii*.

2. Larvae were exposed to sudden salinity changes and stimulated with various intensities of 500-nm light in the horizontal plane. Although the pattern of phototaxis of larvae exposed to 40‰ was unchanged from that at 20‰ (acclimation salinity), the level of positive phototaxis to higher intensities was significantly greater and the level of negative phototaxis to low intensities significantly lower at 40‰. Exposure to low salinity sea water (5‰) generally reverses the sign of phototaxis, since a significantly higher level of negative phototaxis and lower level of positive phototaxis occurs at light intensities above 10^{-2} Wm^{-2}.

3. The minimum amount of salinity decrease from the acclimation salinity that induces a reversal in phototactic sign from positive to negative phototaxis at 0.19 Wm^{-2} ranges from 1 to 2‰, and appears to be independent of acclimation salinity and developmental stage. Total recovery of a positive phototaxis occurs in approximately 5.5 minutes for both zoeal stages, with a 50% return apparent in 1.5–3.2 minutes.

4. Larvae stimulated from above with light of 0.15 Wm^{-2} or maintained in darkness in a vertical column exhibited salinity-dependent as well as age-dependent vertical distributions. At each of the seven test salinities (from 5 to 40‰), Stage IV zoeae maintained a lower position in the column than Stage I.

5. Stage I had similar vertical distributions in darkness and overhead light. At salinities below the acclimation salinity larvae moved downward due to a positive geotaxis and negative phototaxis. Upon exposure to higher salinities, an upward movement due to a negative geotaxis and positive phototaxis occurs. Phototaxis, however, is the dominant behavioral response in light.

6. Stage IV zoeae migrate down in overhead light and darkness upon a decrease in salinity. The dominant behavioral response is a positive geotaxis. With an increase in salinity, as ascent only occurs under overhead light, indicating movement results from a positive phototaxis.

7. Anesthetized larvae sink in both high and low salinity water. Thus, the ascent in high salinities does not result from floating due to the increased density of the water. A comparison of descent rates by anesthetized and unanesthetized larvae in 0.15 Wm^{-2} light directed from above and in low salinity water indicates that the normal descent results primarily from active downward swimming, although some larvae exhibit passive sinking.

8. These behavioral responses to increases and decreases in salinity can act as a negative feedback system to keep larvae within the region of acclimatization salinity water in the vertical water column.

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